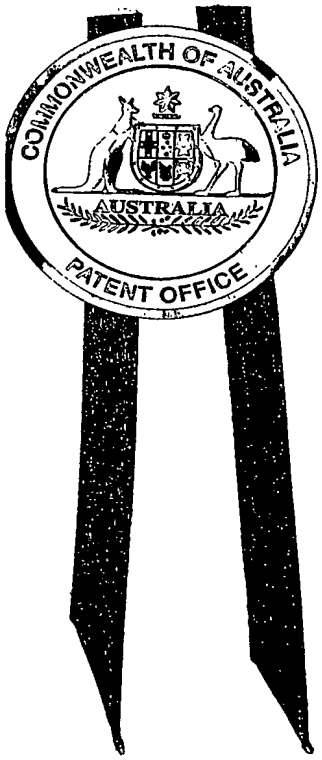
PCT/AU2004/001368

Patent Office
Canberra

I, JULIE BILLINGSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003905512 for a patent by THE UNIVERSITY OF WESTERN SYDNEY as filed on 07 October 2003.



WITNESS my hand this
Eighteenth day of October 2004

JULIE BILLINGSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

S&F Ref: 650375

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

Sequence Selective Compounds

Name and Address of Applicant:

The University of Western Sydney,
Frogmore House, Great Western Highway,
Werrington, New South Wales, 2747, Australia

Names of Inventors:

Janice Aldrich-Wright and David Jaramillo and Warren Howard and Craig Brodie

This invention is best described in the following statement:

SEQUENCE SELECTIVE COMPOUNDS

Technical Field

The present invention relates to sequence selective compounds for targeting therapeutic or diagnostic groups to polynucleotides. More particularly, the present invention relates to sequence selective targeting of metallo complexes, such as metallodrugs and metallodiagnostics, to polynucleotides.

Background of the Invention

Cisplatin is a metallo anticancer drug which stops replication within cells by binding irreversibly to nitrogen (N7), of guanine (G) and adenine (A), and forming intrastrand and interstand cross-links in the major groove of DNA. However, cisplatin binds indiscriminately and also binds with macromolecules other than DNA. This indiscriminate binding can lead to adverse effects in healthy cells. Cisplatin is currently used to treat testicular, ovarian, bladder, head and neck, lung and cervical cancers. However, a drawback of cisplatin is that many human cancer cells have a natural resistance to cisplatin, and those that can be treated may later develop resistance to the drug. In addition, treatment with cisplatin may produce severe side effects in patients, including nephrotoxicity, neurotoxicity, ototoxicity, impairment of sex hormone production and psychosexual difficulties as well as nausea and hair loss. Second generation platinum drugs (such as carboplatin, ZD0473, oxaliplatin) have been developed, however, like cisplatin, they cause indiscriminate, irreversible damage and disadvantageously may have similar negative side effect profiles.

Farrell, et al, *Inorg Chem.*, 38, (1999), 3535 describe metallodrugs based on cisplatin but having two or three platinum centres linked by an alkyl chain. These compounds have been shown to cross the cell membrane and bind to DNA and are active in some *cisplatin* resistant cell lines. However, like cisplatin, these compounds are not sequence specific.

Brabec and co-workers (*Biochemistry*, 2000, 39, 12639-12649; *Eur. J. Biochem.* 1999, 266) have prepared compounds in which *cisplatin* is attached to the minor groove binding molecule, distamycin. However, whilst distamycin has an affinity for sequences in the minor groove, it is not sequence selective. Moreover, in those compounds the coupling of the platinum moiety to the very end of the distamycin restricts the binding

interaction of both groups and neither the distamycin nor the platinum are in a position to optimise their binding interaction.

The present invention relates to compounds in which a metallo complex, such as a metallodrug or metallo-diagnostic compound, is attached to a sequence selective polyamide(s) as a means of selectively targeting the metallo complex to a particular sequence of interest.

Summary of the Invention

According to a first aspect of the invention there is provided a compound of formula (1)

$$[M^1\text{-}T^1]_a\text{-}[P^1\text{-}T^2\text{-}M^2]_b\text{-}[T^3\text{-}P^2]_c \qquad (1)$$

or a salt thereof,

wherein

M^1 and M^2 are the same or different and are each a metal coordination complex, wherein at least one of M^1 and M^2 is capable of interacting with a major groove or minor groove of a polynucleotide;

P^1 and P^2 are the same or different and are each a pyrrole-imidazole polyamide;

T^1, T^2 and T^3 are the same or different and are each a linker group;

a is 0 or 1;

b is an integer selected from 1, 2, 3, 4 and 5; wherein when b is an integer greater than 1, each P^1, each T^2 and each M^2 may be the same or different; and

c is 0 or 1.

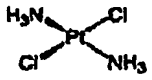
The compound of formula (1) may be charged or uncharged. In one embodiment the compound of formula (1) is charged.

In one embodiment of the first aspect of the invention a = 0, b = 1, and c = 0. In another embodiment, a = 0, b = 1, and c = 1. In another embodiment a = 1, b = 1, and c = 0.

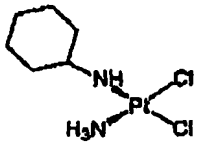
At least one of M^1 and M^2 may bind covalently to a major groove or a minor groove of a polynucleotide. For example, at least one of M^1 and M^2, such as M^2, may bind covalently to a major groove.

In one embodiment, at least one of M^1 and M^2 may be a therapeutic agent. The therapeutic agent may be a platinum complex. For example, the platinum complex may be of the general formula [Pt(diammine)L_2], where each L is a suitable monodentate ligand, or two L taken together is a suitable bidentate ligand. For example, the platinum complex may be of the type [Pt(diammine)Cl_2], such as:

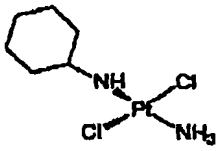
Cisplatin



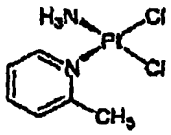
Transplatin



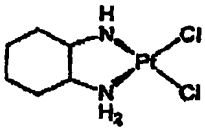
cis-ammine(cyclohexylamine)-dichloro platinum(II)



trans-ammine(cyclohexylamine)-methylpyridine) platinum(II)



cis-amminedichloro(2- dichloro platinum(II)



cis-amminedichloro(1,2-diaminocyclohexane) platinum(II)

In one embodiment, at least one of M^1 and M^2 may be a reporter group. The reporter group may comprise a fluorescent group, or a group capable of becoming fluorescent upon binding, eg, intercalating to, a polynucleotide such as DNA. For example, the reporter group may comprise a rhenium complex or a ruthenium complex. For example, the ruthenium complex may be of the general formula $[Ru(L\text{-}L)(L'\text{-}L')(L''\text{-}L'')]^{2+}$, where L-L, L'-L', and L"-L" respectively may be the same or different and represent a bidentate ligand, or where (L-L)(L'-L') together represent a tetradentate ligand, or where any of L-L, L'-L', and L"-L" may represent two suitable monodentate ligands. Examples of bidentate ligands include 2,2'-bipyridine, 5,5'-dimethyl-2,2'-bipyridine, 4,4'-dimethyl-2,2'-bipyridine, dipyrido[*3,2-a: 2'3'-c*]phenazine, dipyrido[3,2-a:2',3'-c](6,7,8,9-tetrahydro)phenazine dipyrido[*3,2-d:2'3'-f*]quinoxaline, 9,10-phenanthrenenequinone diamine, 2,2':6',2"-terpyridine, 1,10-phenanthroline, substituted 1,10-phenanthroline, including methylated derivatives, 4,7-diamino-1,10-phenanthroline; 3,8-diamino-1,10-phenanthroline; 4,7-diethylenediamine-1,10-phenanthroline; 3,8-

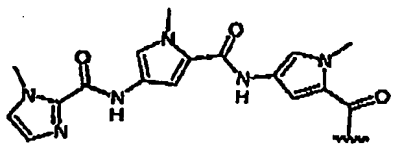
diethylenediamine-1,10-phenanthroline; 3,8-dinitro-1,10-phenanthroline; 4,7-diphenyl-1,10-phenanthroline; or 3,8-diphenyl-1,10-phenanthroline.

In another embodiment, at least one of L-L, L'-L', and L"-L", or (L-L)(L'-L') together, may be substituted with a fluorescent group or a group capable of becoming fluorescent upon binding to DNA, or an intercalator group. The intercalator group may also be a which fluoresces upon intercalating to DNA. Examples of fluorescent groups include dppz (dipyrido[*3,2-a:2'3'-c*]phenazine), dpqC (dipyrido[3,2-a:2',3'-c](6,7,8,9-tetrahydro)phenazine), and dpq (dipyrido[*3,2-d:2'3'-f*]quinoxaline).

In accordance with the present invention, the pyrrole-imidazole polyamide(s) comprise a plurality of heterocyclic rings selected from the group consisting of optionally substituted Im (where "Im" is N-methylimidazole), optionally substituted Py (where "Py" is N-methylpyrrole) and optionally substituted Hp (where "Hp" is 3-hydroxy N-methylpyrrole). Respective heterocyclic ring(s) may be substituted, for example, with any one or more groups selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{6-10} aryl groups. The heterocyclic groups in a pyrrole-imidazole polyamide may be the same or different and may be arranged in any order. Examples of pyrrole-imidazole polyamides have been described in US 6,472,537 to Baird and Dervan, entitled "Polyamides for binding in the minor groove of double stranded DNA"; and *Bioorganic and Medicinal Chemistry*, 9, (2001) 2215-2235, the entire contents of which are incorporated herein by cross-reference.

The number of heterocyclic moieties in each pyrrole-imidazole-polyamide may be from 2 to 10. For example, the number of heterocyclic moieties may be 2, 3, 4, 5, 6, 7, 8, 9 or 10. For instance, the pyrrole-imidazole polyamide may be a triamide or tetraamide comprising 3 or 4 heterocyclic groups, respectively, selected from optionally substituted Im, optionally substituted Py and optionally substituted Hp, where the heterocyclic groups may be in any nominated order. The number and order of Im, Py and Hp groups in a pyrrole-imidazole polyamide may be chosen so as to produce a polyamide selective for a polynucleotide sequence of interest.

For example, the pyrrole-imidazole polyamide may be Im/Py/Py:



The linker groups (T^1, T^2, T^3) may comprise at least one functional group suitable for coordinating to a metal ion such as Pt, Ru, Rh. Optionally, the linker groups (T^1, T^2, T^3) may comprise at least one functional group suitable for allowing the linker group to be bonded, eg, covalently bonded, to a pyrrole-imidazole polyamide. For example, in one embodiment the linker group may have the formula (2)

$$-Y^1-(CR^1R^2-X)_n-Y^2- \quad (2)$$

wherein

at least one of Y^1 and Y^2 is a group capable of coordinating to a metal. For example, Y^1 and Y^2 may individually be a substituted or unsubstituted amino group such as $-N(R^3)_2$ where R^3 is hydrogen, alkyl, cycloalkyl, aryl or heteroaryl; cyclohexylamine; S; O; a heteroaromatic group such as pyridyl;

R^1 and R^2 are independently selected from hydrogen, C_{1-6} alkyl, C_{6-10} aryl, C_{3-6} cycloalkyl, C_{3-6} heterocycloalkyl, and C_{6-10} heteroaryl;

X is selected from NH, O, S, spermidine, or is absent; or

a (CR^1R^2-X) group taken together may be C_{3-6} cycloalkyl, C_{3-6} heterocycloalkyl, C_{6-10} aryl or C_{6-10} heteroaryl; and

n is an integer selected from 1 to 20, wherein when n is an integer greater than 1, each (CR^1R^2-X) group may be the same or different.

For example, n may be an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

For example, in one embodiment the linker group may have the formula (2a)

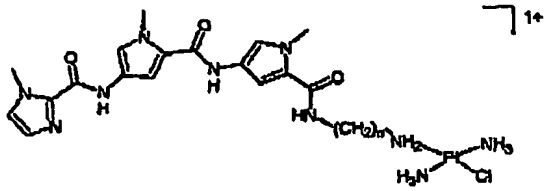
$$-NH_2-(CR^1R^2-X)_n-NH- \quad (2a)$$

where R^1, R^2, X and n are as defined above, and where

the $-NH_2$ moiety is capable of coordinating to a metal ion, such as Pt, Ru, Rh; and the –NH- group forms a covalent bond to a pyrrole-imidazole polyamide, eg, as part of an amide bond.

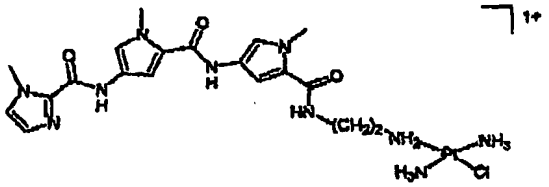
For example, when R^1 and R^2 are hydrogen and X is absent, the linker group may be an alkylenediamine [$-NH_2-(CH_2)_n-NH-$] such as a C_{1-10} alkylenediamine, C_{1-8} alkylenediamine, C_{1-6} alkylenediamine, C_{1-4} alkylenediamine, C_{1-2} alkylenediamine, etc. For example, the alkylenediamine may be methylenediamine, 1,2-ethylenediamine, 1,3-propylenediamine, 1,4-butylenediamine, 1,5-pentylenediamine, or 1,6-hexylenediamine. As another example, when R^1 and R^2 are H, and X is O or absent, the linker group may be $NH_2-CH_2CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2CH_2-NH_2$.

In one embodiment of the invention the compound of formula (1) is "*trans-Im/Py/Py-Pt*":

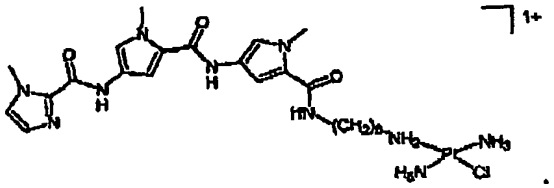


where n is an integer selected from 1, 2, 3, 4, 5, 6, 7,8, 9, and 10, or a salt thereof.

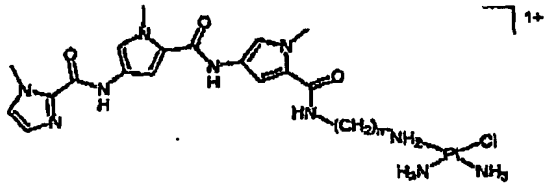
For example, the compound of formula (1) may be



or

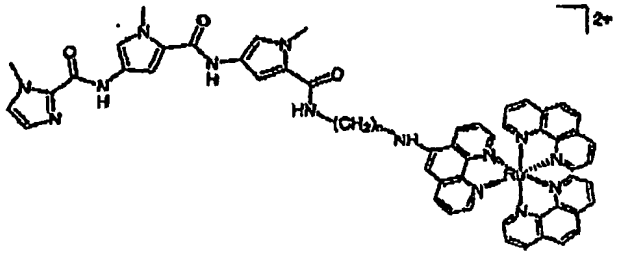


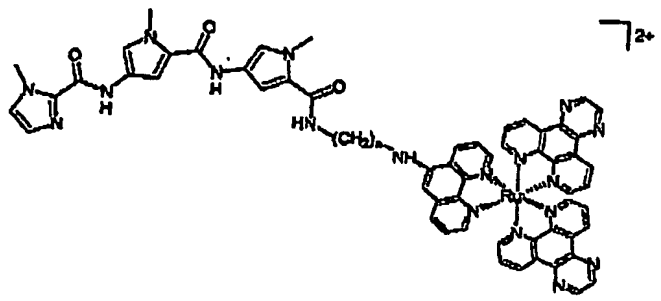
In another embodiment the compound of formula (1) is "*cis-Im/Py/Py-Pt*":

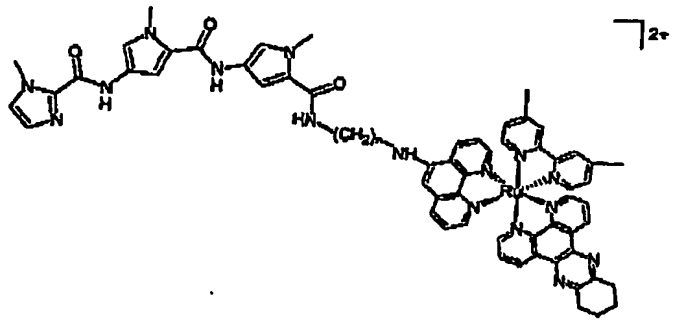


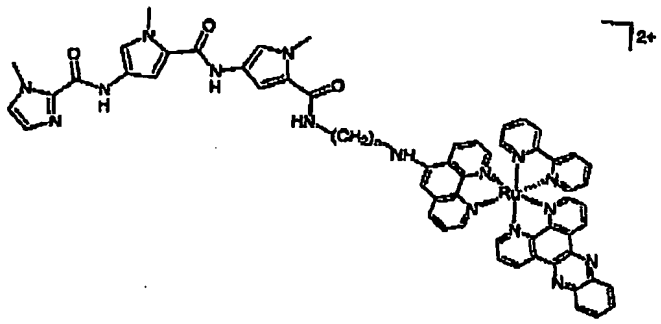
where n is an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, or a salt thereof.

In another embodiment of the invention the compound of formula (1) may be selected from the group consisting of:

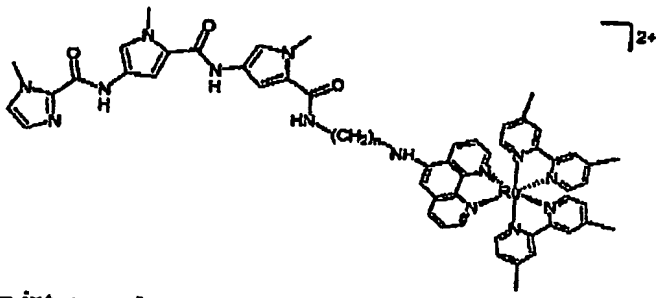






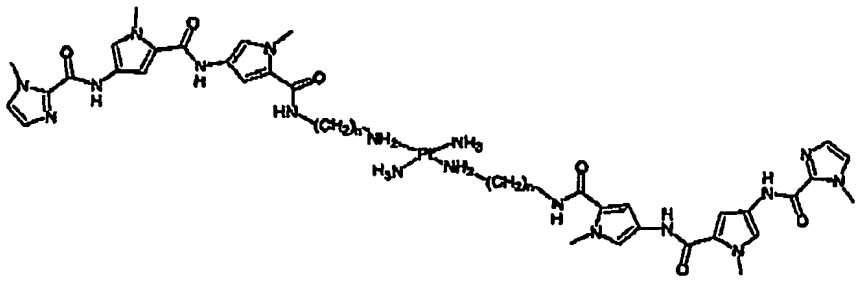


and



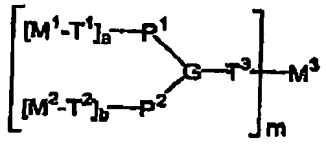
where n is an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, or a salt thereof.

In one embodiment of the invention the compound of formula (1) is "*trans-Im/Py/Py-Pt-Py/Py/Im*":



where n is an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, or a salt thereof.

According to a second aspect of the invention there is provided a compound of formula (3)



(3)

where

M^1, M^2, M^3 are the same or different and are each a metal coordination complex, wherein at least one of M^1, M^2 and M^3 is capable of interacting with a major groove or minor groove of a polynucleotide;

T^1, T^2 and T^3 are the same or different and are each a linker group;

P^1 and P^2 are the same or different and are each a pyrrole-imidazole polyamide;

G is a connecting group;

a and b are independently selected from 0 and 1; and

m is 1, 2, 3 or 4.

In one embodiment, m is 1. In another embodiment, m is 2.

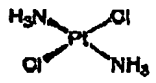
The compound of formula (3) may be charged or uncharged. In one embodiment the compound of formula (3) is charged.

In one embodiment of the second aspect of the invention a = 0, b = 1, m = 1. In another embodiment, a = 1, b = 0, and m = 1. In another embodiment a = 1, b = 1, and m = 1. In another embodiment a = 0, b = 1, and m = 2. In another embodiment a = 1, b = 0, and m = 2. In a further embodiment, a and b are not both 1.

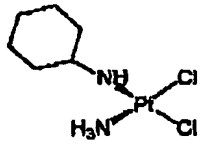
At least one of M^1, M^2 and M^3 may bind covalently to a major groove or a minor groove of a polynucleotide. For example, at least one of M^1, M^2 and M^3, such as M^3, may bind covalently to a major groove.

In one embodiment, at least one of M^1, M^2 and M^3 may be a therapeutic agent. The therapeutic agent may be a platinum complex. For example, the platinum complex may be of the general formula [Pt(diammine)L_2], where each L is a suitable monodentate ligand, or two L taken together is a suitable bidentate ligand. For example, the platinum complex may be of the type [Pt(diammine)Cl_2], such as:

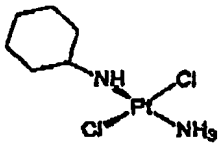
Cisplatin



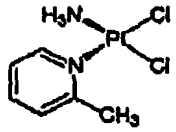
Transplatin



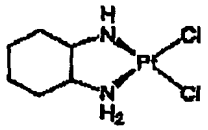
cis-ammine(cyclohexylamine)-dichloro platinum(II)



trans-ammine(cyclohexylamine)-methylpyridine) platinum(II)



cis-amminedichloro(2- dichloro platinum(II)



cis-amminedichloro(1,2-diaminocyclohexane) platinum(II)

In one embodiment, at least one of M^1, M^2 and M^3 may be a reporter group, such as a reporter group comprising a fluorescent group or group capable of becoming fluorescent upon binding DNA. For example, the reporter group may comprise a rhenium complex or a ruthenium complex. For example, the ruthenium complex may be of the general formula $[Ru(L\text{-}L)(L'\text{-}L')(L''\text{-}L'')]^{2+}$, where L-L, L'-L', and L"-L" respectively may be the same or different and represent a bidentate ligand, or where (L-L)(L'-L') together represent a tetradentate ligand, or where any of L-L, L'-L', and L"-L" may represent two suitable monodentate ligands. Examples of bidentate ligands include 2,2'-bipyridine, 5,5'-dimethyl-2,2'-bipyridine, 4,4'-dimethyl-2,2'-bipyridine, dipyrido[*3,2-a: 2'3'-c*]phenazine, dipyrido[3,2-a:2',3'-c](6,7,8,9-tetrahydro)phenazine dipyrido[*3,2-d:2'3'-f*]quinoxaline,

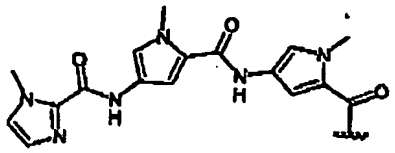
9,10-phenanthrenequinone diamine, 2,2':6',2"-terpyridine, 1,10-phenanthroline, substituted 1,10-phenanthroline, including methylated derivatives, 4,7-diamino-1,10-phenanthroline; 3,8-diamino-1,10-phenanthroline; 4,7-diethylenediamine-1,10-phenanthroline; 3,8-diethylenediamine-1,10-phenanthroline; 3,8-dinitro-1,10-phenanthroline; 4,7-diphenyl-1,10-phenanthroline; or 3,8-diphenyl-1,10-phenanthroline.

In another embodiment, at least one of L-L, L'-L', and L"-L", or (L-L)(L'-L') together, may be substituted with a fluorescent group or group capable of becoming fluorescent upon binding to a polynucleotide such as DNA, or an intercalator group. The intercalator group may also be a fluorescent group or group capable of becoming a fluorescent group. Examples of fluorescent groups include dppz (dipyrido[*3,2-a:2'3'-c*]phenazine), dpqC (dipyrido[3,2-a:2',3'-c](6,7,8,9-tetrahydro)phenazine), and dpq (dipyrido[*3,2-d:2'3'-f*]quinoxaline).

In accordance with the present invention, the pyrrole-imidazole polyamide(s) comprise a plurality of heterocyclic rings selected from the group consisting of optionally substituted Im (where "Im" is N-methylimidazole), optionally substituted Py (where "Py" is N-methylpyrrole) and optionally substituted Hp (where "Hp" is 3-hydroxy N-methylpyrrole). Respective heterocyclic ring(s) may be substituted, for example, with any one or more groups selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{6-10} aryl groups. The heterocyclic groups in a pyrrole-imidazole polyamide may be the same or different and may be arranged in any order. Examples of pyrrole-imidazole polyamides have been described in US 6,472,537 to Baird and Dervan, entitled "Polyamides for binding in the minor groove of double stranded DNA"; and *Bioorganic and Medicinal Chemistry*, 9, (2001) 2215-2235, the entire contents of which are incorporated herein by cross-reference.

The number of heterocyclic moieties in each pyrrole-imidazole-polyamide may be from 2 to 10. For example, the number of heterocyclic moieties may be 2, 3, 4, 5, 6, 7, 8, 9 or 10. For instance, the pyrrole-imidazole polyamide may be a triamide or tetraamide comprising 3 or 4 heterocyclic groups, respectively, selected from optionally substituted Im, optionally substituted Py and optionally substituted Hp, where the heterocyclic groups may be in any nominated order. The number and order of Im, Py and Hp groups in a pyrrole-imidazole polyamide may be chosen so as to produce a polyamide selective for a polynucleotide sequence of interest.

For example, the pyrrole-imidazole polyamide may be Im/Py/Py:



The linker groups (T^1, T^2, T^3) may comprise at least one functional group suitable for coordinating to a metal ion such as Pt, Ru, Rh. Optionally, the linker groups (T^1, T^2, T^3) may comprise at least one functional group suitable for allowing the linker group to be bonded, eg, covalently bonded, to a pyrrole-imidazole polyamide. For example, in one embodiment the linker group may have the formula (2)

$$-Y^1-(CR^1R^2-X)_n-Y^2- \quad (2)$$

wherein

at least one of Y^1 and Y^2 is a group capable of coordinating to a metal. For example, Y^1 and Y^2 may individually be a substituted or unsubstituted amino group such as $-N(R^3)_2$ where R^3 is hydrogen, alkyl, cycloalkyl, aryl or heteroaryl; cyclohexylamine; S; O; a heteroaromatic group such as pyridyl;

R^1 and R^2 are independently selected from hydrogen, C_{1-6} alkyl, C_{6-10} aryl, C_{3-6} cycloalkyl, C_{3-6} heterocycloalkyl, and C_{6-10} heteroaryl;

X is selected from NH, O, S, spermidine, or is absent; or

a (CR^1R^2-X) group taken together may be C_{3-6} cycloalkyl, C_{3-6} heterocycloalkyl, C_{6-10} aryl or C_{6-10} heteroaryl; and

n is an integer selected from 1 to 20, wherein when n is an integer greater than 1, each (CR^1R^2-X) group may be the same or different.

For example, n may be an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

For example, in one embodiment the linker group may have the formula (2a)

$$-NH_2-(CR^1R^2-X)_n-NH- \quad (2a)$$

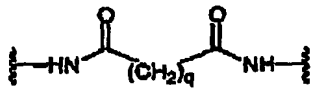
where R^1, R^2, X and n are as defined above, and where

the $-NH_2$ moiety is capable of coordinating to a metal ion, such as Pt, Ru, Rh; and the –NH- group forms a covalent bond to a pyrrole-imidazole polyamide, eg, as part of an amide bond.

For example, when R^1 and R^2 are hydrogen and X is absent, the linker group may be an alkylenediamine $[-NH_2-(CH_2)_n-NH-]$ such as a C_{1-10} alkylenediamine, C_{1-8} alkylenediamine, C_{1-6} alkylenediamine, C_{1-4} alkylenediamine, C_{1-2} alkylenediamine, etc. For example, the alkylenediamine may be methylenediamine, 1,2-ethylenediamine, 1,3-propylenediamine, 1,4-butylenediamine, 1,5-pentylenediamine, or 1,6-hexylenediamine.

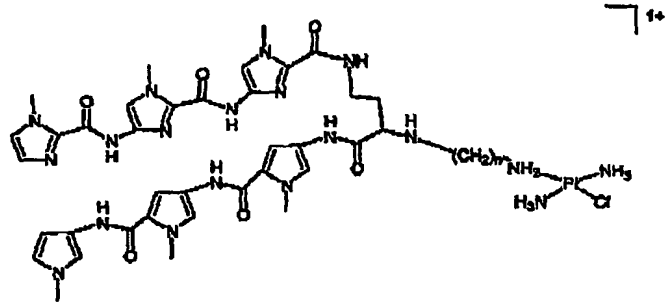
As another example, when R^1 and R^2 are H, and X is O or absent, the linker group may be NH_2-$CH_2CH_2CH_2$-O-CH_2CH_2-O-CH_2CH_2-O-$CH_2CH_2CH_2$-NH_2.

G may be any suitable group capable of connecting two or more pyrrole-imidazole polyamides. For example, G may connect two pyrrole-imidazole polyamides. For example, G may comprise an alkyl amide or alkyl diamide. For example, G may be



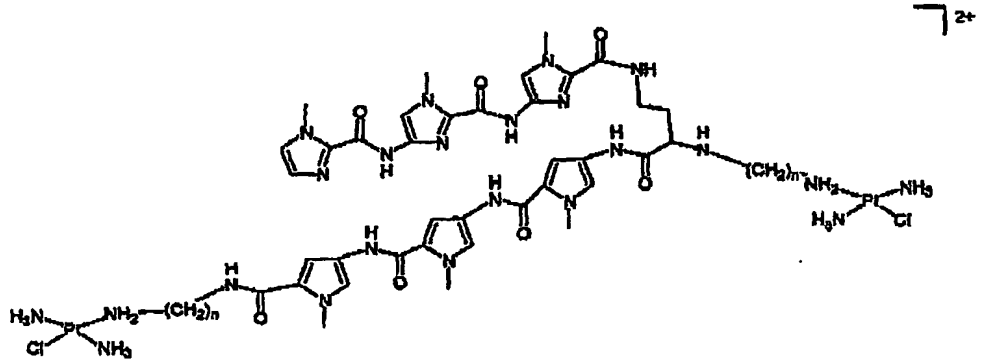
where q is an integer from 1 to 10. For example, q may be 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

In one embodiment the compound of formula (3) may be "*trans-[Im/Im/Im-G-Py/Py/Py]Pt*":



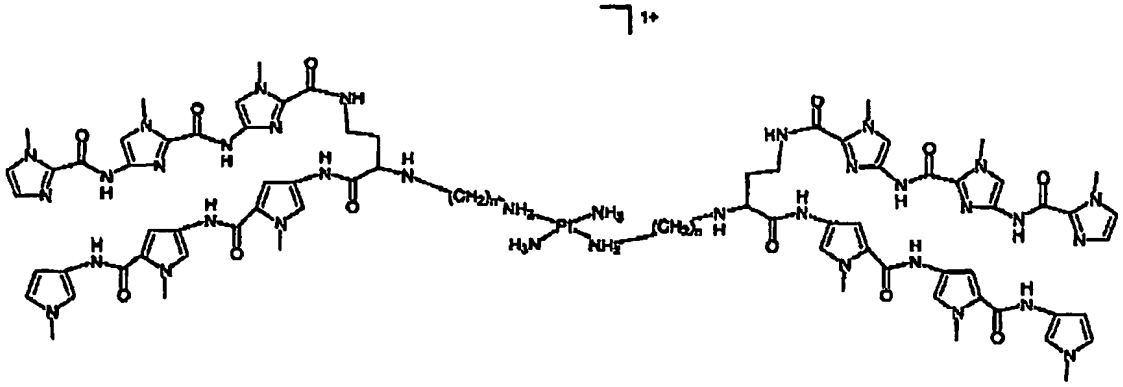
where n is an integer selected from 1, 2, 3, 4, 5 and 6, or a salt thereof.

In another embodiment the compound of formula (3) may be



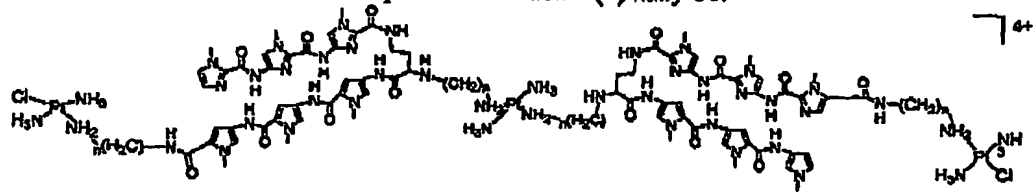
where n is an integer selected from 1, 2, 3, 4, 5 and 6, or a salt thereof.

In another embodiment, the compound of formula (3) may be:



where n is an integer selected from 1, 2, 3, 4, 5 and 6, or a salt thereof.

In another embodiment the compound of formula (3) may be:



where n is an integer selected from 1, 2, 3, 4, 5 and 6, or a salt thereof.

The choice and combination of Im, Py and Hp groups in the respective polyamide chains of compounds of formulae (1) and (3) determine sequence selectivity of the compound. The value of a, b and m controls the overall charge of the compound.

For example, the combination of polyamides Im/Im/Im and Py/Py/Py would be selective for a central core of 5'-CCC-3' in a polynucleotide. As a further example, the combination of polyamides Hp/Py/Hp/Py and Py/Hp/Py/Hp would be selective for a core sequence of 5'-TATA-3'.

In accordance with a fourth aspect of the invention there is provided a compound according to formula (4)

$$[P^1]_e\text{-}[T^1\text{-}P^2]_f\text{-}[T^2]_g \qquad (4)$$

or a salt thereof,

wherein

P^1 and P^2 are the same or different and are each a pyrrole-imidazole polyamide;

T^1 and T^2 are the same or different and are each a linker group;

e is 0 and 1;

f is an integer selected from 1, 2, and 3; wherein when b is an integer greater than 1, each T^1 and each P^2 may be the same or different; and

g is 0 or 1.

Compounds of formula (4) may be substituted with at least one other group, such as a therapeutic group, a diagnostic agent, a metal coordination complex, a fluorophore.

In accordance with a sixth aspect of the invention there is provided a process for preparing compounds of formula (1) or formula (3) comprising reacting a compound of formula (4) with a metal coordination complex to produce a compound of formula (1) (or formula (3).

In one embodiment of the fifth aspect of the invention, compounds of formula (4) may be concatenated before reacting with a coordination metal complex.

In accordance with a sixth aspect of the invention there is provided a pharmaceutical composition comprising a compound according to the first aspect of the invention together with a pharmaceutically acceptable diluent, adjuvant or carrier.

In accordance with a seventh aspect of the invention there is provided a pharmaceutical composition comprising a compound according to the second aspect of the invention together with a pharmaceutically acceptable diluent, adjuvant or carrier.

In accordance with an eighth aspect of the invention there is provided a method of targeting a therapeutic agent(s) and/or a reporter group(s) to a sequence in a polynucleotide comprising contacting biological material suspected of containing said sequence with a compound of formula (1) or formula (3).

In one embodiment of the eighth aspect of the invention, the method comprises contacting the biological material *in vivo*. In another embodiment of the eighth aspect of the invention, the method comprises obtaining a sample of biological material from an organism and contacting said sample with a compound of formula (1) or formula (3) *in vitro*.

In accordance with a ninth aspect of the invention there is provided a method of treating a disease comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to the first or second aspect of the invention or a pharmaceutical composition according to the sixth or seventh aspect of the invention.

With reference to the ninth aspect of the invention, in one embodiment the disease may be HIV or a proliferative disease, such as cancer. For example, the cancer may be selected from breast cancer, ovarian cancer, lung cancer (eg small cell carcinoma), oesophageal cancer, testicular cancer, cervical cancer, bladder cancer, thyroid cancer, neoblastoma, leukemia, and osteogenic sarcoma.

In accordance with a tenth aspect of the invention there is provided a method of diagnosis comprising contacting a biological sample with a diagnostically effective amount of a compound of the first or second aspect of the invention or a salt thereof, or a pharmaceutical composition according to the sixth or seventh aspect of the invention. In one embodiment the method comprises contacting said biological sample *in vivo*, for example, by administering to said mammal a diagnostically effective amount of said compound or composition. In another embodiment the method comprises obtaining a biological sample from said mammal and contacting said sample with a diagnostically effective amount of said compound or composition.

The mammal may be human, non-human primate, murine, bovine, ovine, equine, caprine, leporine, avian, feline or canine.

Definitions

The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for a better understanding of the following description.

In the context of this specification, the term "comprising" means "including principally, but not necessarily solely". Furthermore, variations of the word "comprising", such as "comprise" and "comprises", have correspondingly varied meanings.

In the context of this invention, the term "metal coordination complex" should be understood to mean that there are sufficient ligands or donor groups about the coordinating metal sufficient to complete the coordination sphere. Ligands coordinating the metal may be mondentate, or multidentate such as bidentate, tridentate, or tetradentate, as appropriate. Suitable ligands for a specific metal are known generally to those skilled in the art. Ligands may be coordinated to a metal in any suitable configuration, for example, *cis* or *trans* isomers. The ligands may be (R), (S), Δ, or Λ isomers, as appropriate.

Cisplatin is cis-diamminedichloroplatinum(II).

Transplatin is trans-diamminedichloroplatinum(II).

In this context of this invention, the term "pyrrole-imidazole polyamide" means any polyamide comprising heterocyclic groups selected from optionally substituted N-methylimidazole (abbreviated "Im"), optionally substituted N-methyl-pyrrole

(abbreviated "Py"), and optionally substituted 3-hydroxy N-methylpyrrole (abbreviated "Hp"), wherein the heterocyclic groups may be arranged in any order.

The term "alkyl" as used herein, includes within its meaning monovalent, saturated, straight and branched chain hydrocarbon radicals.

The term "alkenyl" as used herein, includes within its meaning, monovalent, straight and branched chain hydrocarbon radicals having at least one double bond.

The term "alkylene" as used herein, includes within its meaning divalent, saturated, straight chain hydrocarbon radicals.

The term "alkenylene" as used herein, includes within its meaning, divalent, straight chain hydrocarbon radicals having at least one double bond.

The term "alkynylene" as used herein, includes within its meaning, divalent, straight chain hydrocarbon radicals having at least one triple bond.

The term "aryl" as used herein, includes within its meaning monovalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals.

The term "arylene" as used herein, includes within its meaning divalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals.

The term "cycloalkyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals.

The term "cycloalkylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals.

The term "cycloalkenyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having at least one double bond.

The term "cycloalkenylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having at least one double bond.

The term "halo" or "halogen" as used herein, includes fluoro, chloro, bromo and iodo.

The term "heteroaryl" as used herein, includes within its meaning monovalent, single, polynuclear, conjugated and fused aromatic radicals having 1 to 12 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N and S.

The term "heteroarylene" as used herein, includes within its meaning divalent, single, polynuclear, conjugated and fused aromatic radicals having 1 to 12 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N and S.

The term "heterocycloalkyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused radicals wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkenyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals having at least 1 double bond and wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkenylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals having at least one double bond and wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The terms "therapeutically effective amount" and "diagnostically effective amount" as used herein, includes within its meaning a sufficient amount a compound or composition of the invention to provide the desired therapeutic or diagnostic effect. The exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered and the mode of administration and so forth. Thus, it is not possible to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

Brief Description of the Drawings

Figure 1 – Synthetic scheme illustrating preparation of compounds according to the invention.

Figure 2 – DNA melting profiles for *Im/Py/Py-Pt* at 260nm

Figure 3 – Circular Dichroism and Induced Circular Dichroism spectra (240 to 400 nm) for titration of DNA with *Im/Py/Py-Pt*.

Figure 4 – Gel electrophoresis demonstrating binding of *Im/Py/Py-Pt* to a mixture of relaxed and negatively supercoiled pUC19 DNA. Lanes: 0 Control r_b= 0; 1, r_b= 0.008; 2, r_b= 0. 0.016; 3, r_b= 0.025; 4, r_b= 0.033; 5, r_b= 0.041; 6, r_b= 0.049; 7, r_b=0.057; 8, r_b= 0.066; 9, r_b= 0.074.

Detailed Description of the Invention

Compounds having a site-specific DNA binding portion joined to a DNA binding metal complex (such as cisplatin, transplatin, ruthenium complexes, rhenium complexes, etc), may be suitable for use as sequence specific metallodrugs or diagnostic agents. The interaction between the sequence specific portion with the DNA backbone serves to selectively target the DNA binding metal complex to a particular region of the DNA.

Compounds comprising pyrrole-imidazole polyamides and metal complexes such as cisplatin, transplatin, ruthenium complexes or rhenium complexes, may selectively target a polynucleotide by binding to short motifs. For example, the motif may be about 3, 4, or 5 bases to about 30 bases in length, about 7 to about 28 bases, about 9 bases to about 26 bases, about 10 bases to about 24 bases, about 11 bases to about 22 bases, about 12 to about 20 bases, about 14 to about 19 bases, about 16 to about 18 bases in length. The pyrrole-imidazole polyamide chain may be used to target a sequence in the minor groove or major groove of a polynucleotide.

In accordance with the present invention, a pyrrole-imidazole polyamide can be chosen to selectively target a minor groove of a polynucleotide, such as DNA, and allow a therapeutic agent, for example a metal coordination complex such as cisplatin or transplatin, to bind covalently in a minor or major groove of DNA, for example, a major groove of DNA.

Also in accordance with the present invention, a pyrrole-imidazole polyamide chain can be used to selectively target a reporter group, for example a metal coordination complex such as $[Ru(diimine)_3]^{2+}$, to a major groove or minor groove, eg, a minor groove, of DNA.

Compounds of the present invention may also comprise a therapeutic agent and a reporter group. For example, compounds of the present invention may comprise a platinum complex, such as transplatin or cisplatin, as well as a fluorophore, such as a ruthenium complex.

The linker group may be chosen to sufficiently distance the metal coordination complex from the pyrrole-imidazole polyamide to optimise the binding interaction of both the polyamide and the metal coordination complex.

The sequence selective pyrrole-imidazole polyamide chain targets the compound to a selected region of DNA. Specific groups in the polyamide chain may be selected on the basis of the nucleotide sequence of interest. Rules for the design of sequence selective polyamide chains are known to those of skill in the art. For example rules for the design of sequence selective polyamide chains are described in US 6,472,537 to Baird and Dervan, entitled "Polyamides for binding in the minor groove of double stranded DNA";

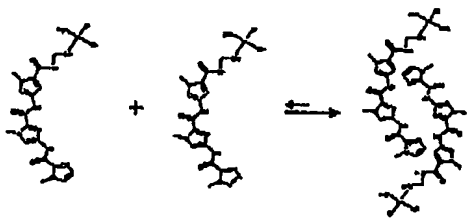
and *Bioorganic and Medicinal Chemistry*, 9, (2001) 2215-2235, the entire contents of which are incorporated herein by cross-reference. For example, it is known that Py/Im targets C-G base pairs; Py/Hp targets A-T base pairs; Hp/Py targets T-A base pairs and Im/Py targets G-C base pairs.

The sequence selective targeting nature of the compounds of the present invention provides the ability to target, for example, a therapeutic or reporter group to any sequence of interest. Such sequences may be associated with a particular disease state, such as cancer, susceptability to a disease, or with infection by an infectious organism, such as HIV. For example, compounds of the invention may be used to specifically deliver a therapeutic agent to a cell infected with HIV, for example, by designing a compound of the invention to target a polynucleotide sequence essential to viral replication. For example, it is known that the Rev Response element (RRE) is an HIV-1 RNA structure essential to viral replication (Frankel et al., *Annu. Rev. Biochem*, 1998, 67, 1-25; Pollard et al, *Annu. Rev. Microbiol.*, 1998, 52, 491-532). Similarly, the transactivation control region of HIV-1 (TAR31) is also believed to be necessary for transcription of full length HIV RNA, such that inhibition of the RNA protein interaction by targeting specific compounds of the invention to a conserved sequence(s) within TAR31 may represent another target for treatment or prevention of HIV infection.

Compounds according to the present invention may bind to a polynucleotide sequence in a 5' to 3' direction or in a 3' to 5' direction.

In the compounds of the invention, a linker group is used to distance the metal coordination complex from the sequence selective polyamide. For example, the length of the linker group can be selected to position the metal coordination complex in a minor or a major groove of DNA.

Compounds of formula (1) and (3) may self-assemble in solution to form oligomeric structures. For example, a self-assembled dimer of a compound of formula (1) [*Im/Py/Py-Pt*] is illustrated below:



[R:\LIBH]A50375v3.doc:lg

Synthesis of Compounds

Compounds of the present invention are adaptable to being prepared in a modular or step-wise fashion. For example, modular molecules, eg, compounds of formulae (1), (2), (3) and (4) may be prepared using the methods disclosed herein or other methods known in the art. The length of the polyamide chain can be selected to bind to a particular nucleotide sequence. Individual modular molecules may be concatenated by attaching to a metal complex, or to other modular molecules, to produce compound(s) having the desired number of pyrrole-imidazole polyamide(s) capable of selectively targeting a particular polynucleotide sequence. For example, compounds of the invention, such as compounds of formulae (1), (3) and (4) comprising pyrrole-imidazole polyamide(s) may be prepared which are capable of binding to a polynucleotide sequence comprising a selected core sequence of about 2, 3, 4, 5, 6, 7, 8, 9, or about 10 base pair groupings.

By way of example, a pyrrole-imidazole polyamide may be coupled to a suitable linker group to produce a modular compound of formula (4). Compound(s) of formula (4) may be concatenated by reacting with a suitable metal coordination complex, such as transplatin, cisplatin, to produce modular compounds of formula (1). Compounds of formula (1) may be reacted, for example, with another compound of formula (4), and so on. This strategy can be adapted to prepare compounds of formula (3) and enables a wide range of modules to be produced. In addition, by coupling the modules with a metal such as *transplatin* the overall charge may be increased which may increase the affinity for negatively charged DNA.

Compounds of formula (1) may be prepared by methods known to those skilled in the art. Suitable methods are generally described, for example, and intermediates thereof are described, for example, in Houben-Weyl, *Methoden der Organischen Chemie;* J. March, *Advanced Organic Chemistry*, 4th Edition (John Wiley & Sons, New York, 1992); D. C. Liotta and M. Volmer, eds, *Organic Syntheses Reaction Guide* (John Wiley & Sons, Inc., New York, 1991); R. C. Larock, *Comprehensive Organic Transformations* (VCH, New York, 1989), H. O. House, *Modern Synthetic Reactions* 2nd Edition (W. A. Benjamin, Inc., Menlo Park, 1972)

Sequence selective chains can be prepared using techniques and reagents well known to those skilled in the art. The synthesis is generally step-wise and based on successive amide coupling reactions. Those skilled in the art will appreciate that automated solid phase coupling methods could also be suitable for synthesising polyamide compounds in accordance with the present invention. Heterocyclic rings of choice, for example N-methyl pyrrole (Py), N-methyl imidazole (Im), 3-hydroxy N-

methyl pyrrole (Hp), etc, can be added as required depending on the nucleotide sequence to be targeted. Suitable protecting groups for use in amide coupling reactions are well know by those in the art and have been described in Greene et al., *Protective Groups in Organic Synthesis*; John Wiley & Sons, 2nd Edition, 1991. Typically, the t-butyl carbamate(BOC) or (FMOC) protecting group may be used to protect terminal amines. For example, FMOC protected diaminobutyric acid (DABA) may be used. Different coupling reagents can be used to help minimise formation of by-products and maximise yields.

Once a suitable sequence selective pyrrole-imidazole polyamide has been prepared, the metal coordination complex may be attached to the linker moiety at a terminal end of the chain. Alternatively, it may be possible to attach the metal coordination complex to the linker moiety before the linker is attached to the polyamide. A further alternative is to attach the metal coordination complex and linker moiety to a heterocyclic ring eg, Im, Py or Hp (or dimer, trimer etc comprising Im, Py, Hp), then subsequently carry out further amide couplings to attach further heterocyclic rings to build up a polyamide chain or desired length.

By varying the ratio of pyrrole-imidazole polyamide to metal coordination complex (eg, 1:1; 2:1, 1:2 etc), it is possible to manipulate the proportion of products formed. The compounds produced may be isolated using methods well known to those skilled in the art, for example, column chromatography and HPLC.

Compounds of formula (1) according to the present invention may bind irreversibly to polynucleotides, eg, DNA, via covalent bonds. Where the compounds are covalently bound to DNA, any testing can be destructive to both the metal complex and the DNA. The nature of the binding of the compounds according to the invention with DNA can be probed using a variety of techniques known to those skilled in the art to characterise the interactions. For example,

(a) DNA-Melting Experiments Monitored by Absorption Spectrophotometry - UV melting experiments can be used to assess the impact of compounds of formula (1) on the thermal stability of DNA duplexes. Techniques are known to those skilled in the art.

By way of example, for the compound *trans-Im/Py/Py-Pt* three 11-mer DNA duplexes can be used:

1) d(CATTGTCAGAC)$_2$ (target site),

2) d(CATTGACAGAC)$_2$ (single mismatch site) and

3) d(CATTGAGAGAC)$_2$ (double mismatch site).

The concentration of the duplex is kept constant, while the complex concentration ratio is be varied with respect to the DNA. The measured differences melting temperature (ΔT_m) between the target site binding to the complexes and that of a single mismatch site and a double mismatch site can be compared with the extent to which the target sequence deviates from the sequence of the match site being correlated with the magnitude of ΔT_m, as illustrated in Figure 2.

(b) Binding Studies by Circular Dichroism (CD) Spectropolarimetry - CD spectropolarimitry may be used to determine the equilibrium constant and hence binding strength of the metallo complexes. One advantage of this method is its sensitivity. Generally, DNA may be titrated into a fixed concentration of a compound of the invention, eg, a compound of formula (1) or (3), resulting in changes to the spectrum. The changes are generally monitored until saturation is reached. The equilibrium constant can be determined using standard techniques, such as Scatchard plot or the McGhee Von Hippel model for analysis.

Preferential binding may be quantified by varying the DNA duplexes. Final CD spectra can be normalised to reflect equimolar concentrations of duplex.

(c) Footprinting Studies The ability of compounds to bind in a sequence specific fashion can be determined though transcription assays. For example, a small fragment of double stranded DNA of specified length can be incubated with a compound of the invention, eg, a compound of formula (1), such as *trans-Im/Py/Py-Pt* etc, then incubated with a cleavage agent under conditions that result in an average of one cleavage event per molecule. The DNA can then purified and analysed by electrophoresis though through 12% denatured polyacrylamide sequencing gels and visualised using techniques known in the art, eg, STAINSALL®, radiolabelled isotopes, etc. If cleavage occurs randomly, the resulting populations of single-stranded DNA fragments will differ in length by a single nucleotide and will appear as a semicontinuous ladder on the gel. However, if a region of DNA is protected from cleavage there will be a gap in the ladder of fragments. The "footprint" can be precisely located, for example, by aligning the gap with a set of Maxam-Gilbert sequencing reactions carried out on the same DNA.

(d) Cell-lines - IC_{50} values (ie, the concentration of compound of the invention required to inhibit cell growth by 50%) can be determined using known techniques.

Pharmaceutical and/or Therapeutic Formulations

In accordance with the present invention, when used for the treatment of disease, compounds of the invention may be administered alone. Alternatively, the compounds may be administered as a pharmaceutical formulation which comprises at least one compound according to the invention. The compound(s) may also be present as suitable pharmaceutically acceptable salts.

By pharmaceutically acceptable salt it is meant those salts which, within the scope of sound medical judgement, are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art.

For instance, suitable pharmaceutically acceptable salts of compounds according to the present invention may be prepared by mixing a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, methanesulfonic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, phosphoric acid, acetic acid, oxalic acid, carbonic acid, tartaric acid, or citric acid with the compounds of the invention. Suitable pharmaceutically acceptable salts of the compounds of the present invention therefore include acid addition salts.

For example, S. M. Berge *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, *66*:1-19. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Representative acid addition salts include acetate, adipate, alginate, ascorbate, asparate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, triethanolamine and the like.

Convenient modes of administration include injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the formulation and/or compound may be coated with a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the therapeutic activity of the compound. The compound may also be administered parenterally or intraperitoneally.

Dispersions of the compounds according to the invention may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, pharmaceutical preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injection include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Ideally, the composition is stable under the conditions of manufacture and storage and may include a preservative to stabilise the composition against the contaminating action of microorganisms such as bacteria and fungi.

In one embodiment of the invention, the compound of the invention may be administered orally, for example, with an inert diluent or an assimilable edible carrier. The analogue and other ingredients can also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into an individual's diet. For oral therapeutic administration, the analogue can be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Suitably, such compositions and preparations may contain at least 1% by weight of active compound. The percentage of the compound of formula (I) in pharmaceutical compositions and preparations can, of course, be varied and, for example, can conveniently range from about 2% to about 90%, about 5% to about 80%, about 10% to about 75%, about 15% to about 65%; about 20% to about 60%, about 25% to about 50%, about 30% to about 45%, or about 35% to about 45%, of the weight of the dosage unit. The amount of analogue in therapeutically useful compositions is such that a suitable dosage will be obtained.

The language "pharmaceutically acceptable carrier" is intended to include solvents, dispersion media, coatings, anti-bacterial and anti-fungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the analogue, use thereof in the therapeutic compositions and

methods of treatment is contemplated. Supplementary active compounds can also be incorporated into the compositions according to the present invention. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the individual to be treated; each unit containing a predetermined quantity of analogue is calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The compound may be formulated for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in an acceptable dosage unit. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

In one embodiment, the carrier may be an orally administrable carrier.

A particularly suitable form of a pharmaceutical composition is a dosage form formulated as enterically coated granules, tablets or capsules suitable for oral administration.

In one embodiment, the compound may be administered by injection. In the case of injectable solutions, the carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by including various anti-bacterial and/or anti-fungal agents. Suitable agents are well known to those skilled in the art and include, for example, parabens, chlorobutanol, phenol, benzyl alcohol, ascorbic acid, thimerosal, and the like. In many cases, it may be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the analogue in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the analogue into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above.

Tablets, troches, pills, capsules and the like can also contain the following: a binder such as gum gragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it can contain, in addition to materials of the above type, a liquid carrier. Various other materials can be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules can be coated with shellac, sugar or both. A syrup or elixir can contain the analogue, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the analogue can be incorporated into sustained-release preparations and formulations.

Preferably, the pharmaceutical composition may further include a suitable buffer to minimise acid hydrolysis. Suitable buffer agent agents are well known to those skilled in the art and include, but are not limited to, phosphates, citrates, carbonates and mixtures thereof.

Single or multiple administrations of the pharmaceutical compositions according to the invention can be carried out. One skilled in the art would be able, by routine experimentation, to determine effective, non-toxic dosage levels of the compound and/or composition of the invention and an administration pattern which would be suitable for treating the disorders or diseases to which the compounds and compositions are applicable.

Further, it will be apparent to one of ordinary skill in the art that the optimal course of treatment, such as the number of doses of the compound or composition of the invention given per day for a defined number of days, can be ascertained using convention course of treatment determination tests.

Generally, an effective dosage per 24 hours may be in the range of about 0.0001 mg to about 1000 mg per kg body weight; suitably, about 0.001 mg to about 750 mg per kg body weight; about 0.01 mg to about 500 mg per kg body weight; about 0.1 mg to about 500 mg per kg body weight; about 0.1 mg to about 250 mg per kg body weight; or about 1.0 mg to about 250 mg per kg body weight. More suitably, an effective dosage per 24 hours may be in the range of about 1.0 mg to about 200 mg per kg body weight; about 1.0 mg to about 100 mg per kg body weight; about 1.0 mg to about 50 mg per kg body

weight; about 1.0 mg to about 25 mg per kg body weight; about 5.0 mg to about 50 mg per kg body weight; about 5.0 mg to about 20 mg per kg body weight; or about 5.0 mg to about 15 mg per kg body weight.

Alternatively, an effective dosage may be up to about 500 mg/m^2. Generally, an effective dosage may be in the range of about 25 to about 500 mg/m^2, about 25 to about 350 mg/m^2, about 25 to about 300 mg/m^2, about 25 to about 250 mg/m^2, about 50 to about 250 mg/m^2, or about 75 to about 150 mg/m^2.

The compounds of the invention may be used in combination with other known treatments, such as surgery and/or therapeutic agents, including chemotherapeutic or radiotherapeutics. When used in the treatment of solid tumours, compounds of the present invention may be administered with chemotherapeutic agents such as: adriamycin, taxol, fluorouricil, melphalan, alpha interferon, COMP (cyclophosphamide, vincristine, methotrexate and prednisone), etoposide, mBACOD (methortrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone), PROMACE/MOPP (prednisone, methotrexate (w/leucovin rescue), doxorubicin, cyclophosphamide, taxol, etoposide/mechlorethamine, vincristine, prednisone and procarbazine), vincristine, vinblastine, angioinhibins, TNP-470, pentosan polysulfate, platelet factor 4, angiostatin, LM-609, SU-101, CM-101, Techgalan, thalidomide, SP-PG and the like. Other chemotherapeutic agents include alkylating agents such as nitrogen mustards including mechlorethamine, melphan, chlorambucil, cyclophosphamide and ifosfamide; nitrosoureas including carmustine, lomustine, semustine and streptozocin; alkyl sulfonates including busulfan; triazines including dacarbazine; ethylenimines including thiotepa and hexamethylmelamine; folic acid analogues including methotrexate; pyrimidine analogues including 5-fluorouracil, cytosine arabinoside; purine analogues including 6-mercaptopurine and 6-thioguanine; antitumour antibiotics including actinomycin D; the anthracyclines including doxorubicin, bleomycin, mitomycin C and methramycin; hormones and hormone antagonists including tamoxifen and cortiosteroids and miscellaneous agents including brequinar.

Examples

The invention will now be described in more detail, by way of illustration only, with respect to the following examples. The examples are intended to serve to illustrate this invention and should not be construed as limiting the generality of the disclosure of the description throughout this specification.

In the present invention one example of a sequence for binding may be d(CATTGTCAGAC)$_2$. Two other 11-mer sequences have been prepared - one with one mismatch and the other with two mismatches. Analogous 18-mers have also been prepared to assess the trinuclear complex, *trans-Im/Py/Py-Pt-Py/Py/Im*. The binding constant of the dimer and trimer may be determined by measuring the change in Circular Dichroism (CD) upon titration of each of the three duplexes. Footprinting experiments may be used to assess the binding fidelity. STAINSALL® can be used for these experiments. STAINSALL® has the advantage that it negates the need to use radiolabelled isotopes. However, radioisotopes may also be used.

The animal tumour, L1210 leukaemia, may be used as a primary screen of newly synthesised compounds in addition to *cisplatin* resistant L1210 cell lines.

Example 1

Synthesis of *Im/Py/Py*

The polyamide *Im/Py/Py* was prepared by a similar method to that of Lown et al.(*J. Org. Chem.*, 1985, 50(20), 374-379). The synthesis of *Im/Py/Py* is shown schematically in Figure 1.

Methyl-4-nitropyrrole-2-carboxylic acid (1).

Acetic anhydride (8ml) and nitric acid (70%, 1.6 ml) were heated to 50 °C for 15 minutes and cooled to room temperature. The solution was then slowly added to a suspension of 1-Methyl-2-pyrrolecarboxylic acid (2.0 g, 0.02 mol) in Ac_2O (12 ml) cooled to –25 °C. The mixture was stirred for 30 min at –15 °C, warmed to room temperature and stirred for another 20 min. The mixture was again cooled to –25 °C and the resulting precipitate collected in a funnel cooled with dry ice. The solid was washed with cold Ac_2O (-25°C), followed by Ac_2O-CCl_4 (1:1, -25 °C), and then CCl_4 and hexane. The yellow solid was dissolved in NaOH (1M) and acidified with HCl to yield the product as a light cream solid which was collected and air-dried. Yield 0.98 g (36%). ^{1}H NMR (DMSO): δ 8.19 (d, 1H, J = 1.8 Hz); 7.23 (d, 1H, J = 2.0 Hz); 3.90 (s, 3H).

Methyl 1-methyl-4-nitropyrrole-2-carboxylate (2).

A solution of H_2SO_4 (0.4 ml) in MeOH (4 ml) was added to compound 1 (0.4 g, 2.35 mmol) and the mixture refluxed for 24hr. Water was added and the mixture extracted with $CHCl_3$. The organic layer was dried (MgSO4), and the solvent evaporated under vacuum. The residue was purified by flash chromatography (100% CH_2Cl_2) to yield the

product as a crystalline solid. Yield 0.33 g (79%). ^{1}H NMR (DMSO): δ 7.57 (d, 1H, J = 2.1 Hz); 7.40 (d, 1H, J = 2.0 Hz); 3.99 (s, 3H); 3.86 (s, 3H, $COOCH_3$).

Methyl 1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxylate (3).

Compound 2 (0.34 g, 1.85 mmol) in methanol (150 ml) and Pd/C (10%, 0.03 g) were stirred under H_2 (1 atm) for 1 hr. The catalyst was removed (celite), and the solvent evaporated to dryness. Diisopropyl ethylamine (1 ml) in THF (5 ml) was added, the solution cooled to –20 °C, and treated with a solution of the acid chloride of 1 (0.31g, refluxed with thionyl chloride) in THF (5 ml). The mixture was allowed to warm to room temperature and stirred for a further 30 min. The solvent was evaporated to dryness, and water (5 ml) added. The solid was collected, and recrystallized by dissolving in hot DMF and precipitating with ethanol. Yield 0.46 g (82%). ^{1}H NMR (DMSO): δ 10.23 (s, 1H, NH); 8.16 (d, 1H, J = 1.9 Hz); 7.52 (d, 1H, J = 2.0 Hz); 7.43 (d, 1H, J = 2.0 Hz); 6.87 (d, 1H, J = 1.9 Hz); 3.93 (s, 3H); 3.86 (s, 3H); 3.73 (s, 3H).

1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxylic acid (4).

Compound 3 (0.10 g, 0.33 mmol), NaOH (0.7M, 2.4 ml) and ethanol (2.4 ml) were refluxed until the solid dissolved. The red solution was cooled and acidified with concentrated HCl to precipitate the product as a yellow solid. Yield 0.09 g, (88%). ^{1}H NMR (DMSO): δ 12.19 (bs, 1H, OH); 10.18 (s, 1H, NH); 8.15 (d, 1H, J = 1.9 Hz); 7.52 (d, 1H, J = 1.9 Hz); 7.36 (d, 1H, J = 1.9 Hz); 6.78 (d, 1H, J = 1.9 Hz); 3.93 (s, 3H); 3.81 (s, 3H).

N-di-tert-butoxycarbonyl-1,2-ethanediamine (en-BOC)

This step was carried out as described by Krapcho et al (Synthetic Communications, 1990, 20(16), 2559-2564). A solution of di-tert-butyl dicarbonate (1.0 g, 4.58 mmol) in CH_2Cl_2 (12 ml) was added over a period of 2.5 hr to a solution of ethylenediamine (2.1g, 35.61 mmol) in CH_2Cl_2 (12 ml), which was cooled in an ice bath. The mixture was allowed to stir at room temperature for 24 hr and the solvent removed under reduced pressure. Water (20 ml) was added and the mixture filtered. The filtrate was extracted with CH_2Cl_2 (3 x 50 ml), the organic layer dried ($MgSO_4$) and the solvent evaporated to yield the product as an oil. Yield 0.64g (87%). NMR ($CDCl_3$): δ 4.93 (bs, 1H, NH-Boc); 3.15 (q, 2H, J_1 = 6.0 Hz, J_2 = 10.8 Hz); 2.77 (t, 2H, J_1 = 6 Hz); 1.42 (s, 9H, Boc); 1.41 (s, 2H, NH_2).

[2-({1-Methyl-4-[(1-methyl-4-nitro-1H-pyrrole-2-carbonyl)-amino]-1H-pyrrole-2-carbonyl}-amino)-ethyl]-carbamic acid tert-butyl ester (5a).

This step was carried out as described by Dervan et al.(*J. Am. Chem. Soc.*, 1992, 114, 8783-8794.)

To a solution of compound 4 (0.07 g, 0.22 mmol), HOBT (0.04 g, 0.26 mmol) and en-Boc (0.04 g, 0.26 mmol) in THF (7 ml) at 0 °C was added EDCI (0.05 g, 0.24 mmol) in CH_2Cl_2 (3 ml). The solution was allowed to warm to room temperature and stirred for 20 hr. The mixture was filtered (celite), and H_2O (15 ml) added. The solution was extracted with $CHCl_3$ (30 ml) and the organic layer dried (MgSO4). The solvent was removed under vacuum and the crude residue purified by flash column chromatography (5% methanol/CH_2Cl_2) to yield the product as a yellow solid. Yield 0.073 g (70%). ^1H NMR (DMSO): δ 10.20 (s, 1H, NH), 8.15 (d, 1H, J = 1.8 Hz), 7.99 (t, 1H, J = 5.4 Hz, NH), 7.55 (d, 1H, J = 1.8 Hz), 7.18 (d, 1H, J = 1.8 Hz), 6.85 (bs, 1H, NH-Boc); 6.83 (d, 1H, J = 1.8 Hz), 3.94 (s, 3H), 3.79 (s, 3H), 3.17 (m, 2H, CH_2), 3.04 (m, 2H, CH_2), 1.37 (s, 9H, $C(CH_3)_3$).

(2-{[1-Methyl-4-({1-methyl-4-[(1-methyl-1H-imidazole-2-carbonyl)-amino]-1H-pyrrole-2-carbonyl}-amino)-1H-pyrrole-2-carbonyl]-amino}-ethyl)-carbamic acid tert-butyl ester (6a).

Compound 5a (0.1 g,0.23 mmol) in methanol (75 ml) was added PtO_2 (0.01g) and the solution hydrogenated at 1atm for 26 hr. Catalyst filtered (celite) and DMF (3ml) added. Methanol removed under vacuum and N-methylimidazole-2-carboxylic acid (0.07g, 0.55 mmol) was added followed by HOBT (0.09g, 0.69 mmol) and TBTU (0.22g, 0.69 mmol). Triethylamine (0.3 ml, 2.3 mmol) was added and the solution stirred for 1 hr. Solvent was removed under vacuum and the residue purified by flash chromatography (3-5 % MeOH/ CH_2Cl_2). Yield 0.1g (42%). ^1H NMR (DMSO): δ 10.47 (s, 1H, NH), 9.93 (s, 1H, NH), 7.98 (t, 1H, NH, J = 6.0 Hz) 7.39 (d, 1H, J = 1.2 Hz), 7.28 (d, 1H, J = 1.5 Hz), 7.18 (d, 1H, J = 1.5 Hz) 7.14 (d, 1H, J = 1.8 Hz), 7.03 (d, 1H, J = 1.2 Hz), 6.88-6.86 (bs, 2H, 1H and NH-Boc), 3.98 (s, 3H), 3.82 (s, 3H), 3.78 (s, 3H), 3.18 (m, 2H, CH_2), 3.04 (m, 2H, CH_2), 1.37 (s, 9H, $C(CH_3)_3$).

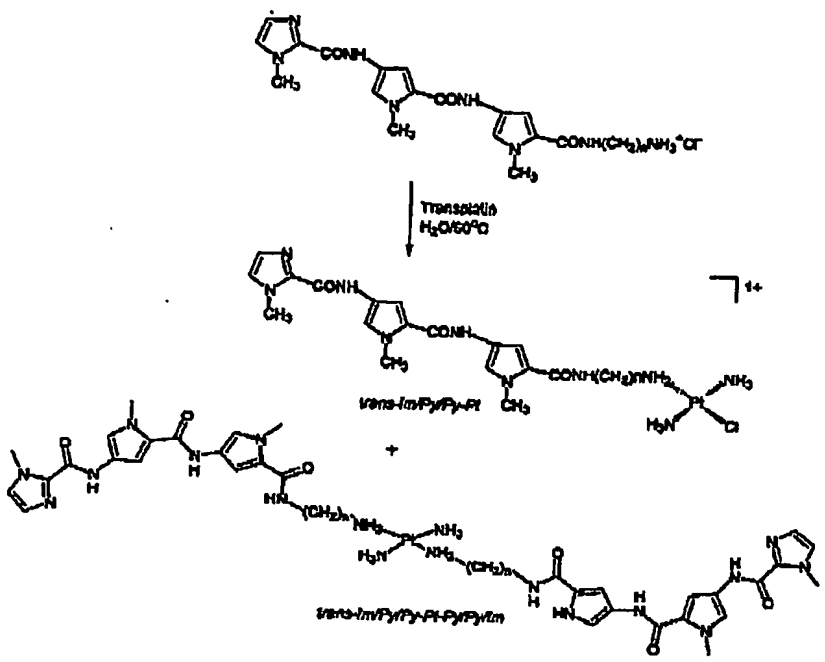
1-Methyl-1H-imidazole-2-carboxylic acid {5-[5-(2-amino-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-ylcarbamoyl]-1-methyl-1H-pyrrol-3-yl}-amide (7a)

Compound 6a (0.29g, 0.06 mmol) and TFA/CH_2Cl_2 (1:1, 2 ml) containing H_2O (40 µl) were stirred at room temperature for 1.5 hr. The solvent was removed under pressure and the residue stirred with DOWEX® 550A OH anion exchange resin (0.05g, 0.17 mmol, washed with MeOH). The solution was decanted and evaporated. $CHCl_3$ (5 ml) was added and the solid collected and dried under vacuum. Yield 0.02g (90%). ^1H NMR (DMSO): δ 10.49 (s, 1H, NH), 9.96 (s, 1H, NH), 8.13 (t, 1H, NH, J = 6.0 Hz) 7.71 (bs, 2H, NH_2), 7.40 (d, 1H, J = 1.5 Hz), 7.28 (d, 1H, J = 1.5 Hz), 7.18 (d, 1H, J = 1.5 Hz) 7.15 (d, 1H, J = 1.8 Hz), 7.04 (d, 1H, J = 1.2 Hz), 6.98 (d, 1H J = 1.5 Hz), 3.98 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.5-3.2 (m, 2H, CH_2), 2.92 (m, 2H, CH_2).

Synthesis of *trans-Im/Py/Py-Pt* and *trans-Im/Py/Py-Pt-Py/Py/Im*

trans-[PtCl(NH_3)$_2$(7a)]Cl

Transplatin (0.10g, 0.34 mmol) and compound 7a (0.14g, 0.34 mmol) in H_2O (45 ml) were refluxed until the mixture dissolved (24hr). The solution was cooled and filtered. The solvent was evaporated and MeOH (10 ml) added. The solid was removed and the filtrate concentrated. CH_2Cl_2 was added (10 ml) and stirred for 30 min. The solid was collected and dried under vacuum The synthesis is represented schematically below. Yield 0.24g (63%). ^1H NMR (DMSO): δ 11.52 (s, 1H, NH), 10.09 (s, 1H, NH), 8.17 (t, 1H, J = 5.4 Hz, NH), 7.84 (bs, 2H, NH_2), 7.56 (d, 1H, J = 1.5 Hz), 7.45 (d, 1H, J = 1.5 Hz), 7.32 (d, 1H, J = 1.5 Hz), 7.29 (s, 1H), 7.20 (d, 1H, J = 1.5 Hz), 7.15 (d, 1H, J = 1.8 Hz), 7.12 (s, 1H), 6.99 (d, 1H, J = 1.8 Hz), 6.95 (s, 1H), 4.00 (s, 3H), 3.90 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.6-3.2 (m, 2H, CH_2), 2.93 (m, 2H, CH_2); MS calculated for $C_{19}H_{30}ClN_{10}O_3Pt^{+1}$ (677.04). Found 677.0



Example 2

DNA Melting Experiments

DNA melting profiles were acquired for *Im/Py/Py-Pt* at 260nm using a Cary 1E recording spectrophotometer equipped with peltier controlled cell holder and cell length of 1 cm. The heating rate in all experiments was 0.5°C/min. Solutions conditions are sodium phosphate (10 mM), edta (1 mM) and NaCl (40 mM) adjusted to pH 7.0. DNA melting curves are shown in Figure 2.

Example 3

CD Titrations

All CD measurements were recorded on a Jasco J-810 CD spectropolarimeter at room temperature and cell length of 1 cm. Titrations were performed by incrementally adding aliquots of *Im/Py/Py-Pt* to a 2600μL solution of 5 μM duplex DNA. After each addition, an average CD spectrum from 240 to 400 nm (20 accumulations) was recorded. DNA concentration was 5 μM. The concentration of *Im/Py/Py-Pt* ranged from 0 to 10 μM. Solution conditions were 10 mM sodium phosphate (pH 7.0) and 40 mM NaCl. CD spectra obtained are shown in Figure 3.

Example 4

Unwinding Experiments

Gel electrophoresis indicates that variable amounts of *Im/Py/Py-Pt* have been bound to a mixture of relaxed and negatively supercoiled pUC19 DNA. The unwinding angle was determined to be 13° for this experiment, which is the same as that reported for *cisplatin* (13°).[7] The plasmid was incubated with *Im/Py/Py-Pt* for 1.5 hr at 37 °C. Lanes: 0 Control r_b= 0; 1, r_b= 0.008; 2, r_b= 0. 0.016; 3, r_b= 0.025; 4, r_b= 0.033; 5, r_b= 0.041; 6, r_b= 0.049; 7, r_b=0.057; 8, r_b= 0.066; 9, r_b= 0.074. The top bands corresponds to the form of nicked plasmid and the bottom bands to the closed, negatively supercoiled plasmid shown in Figure 4.

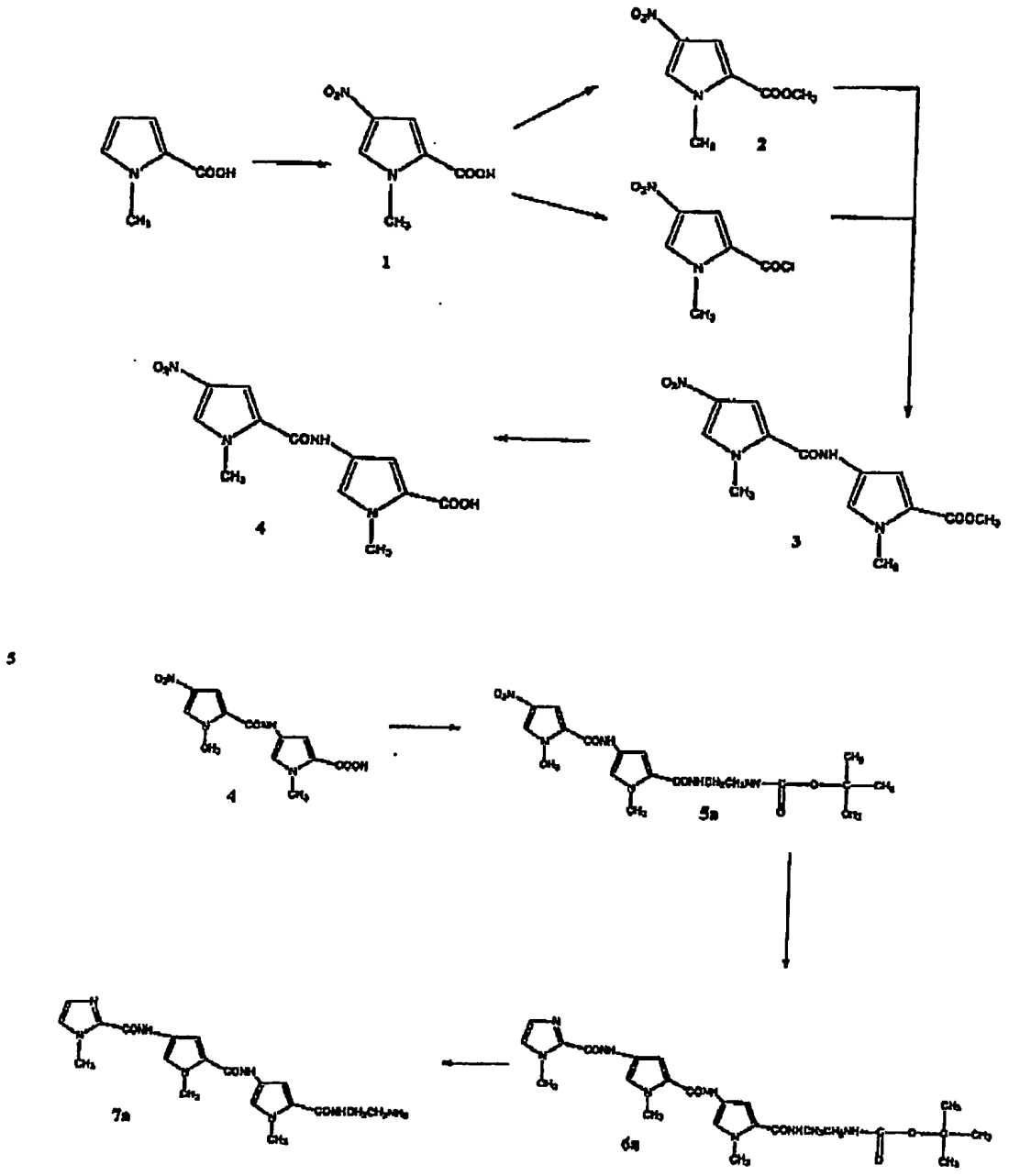
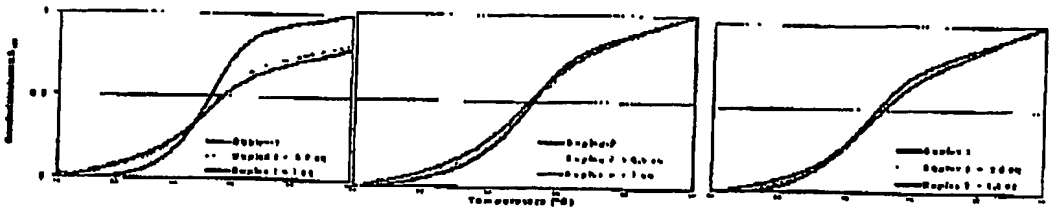


Figure 1



10

Figure 2

CD titration Spectra

ICD titration Spectra

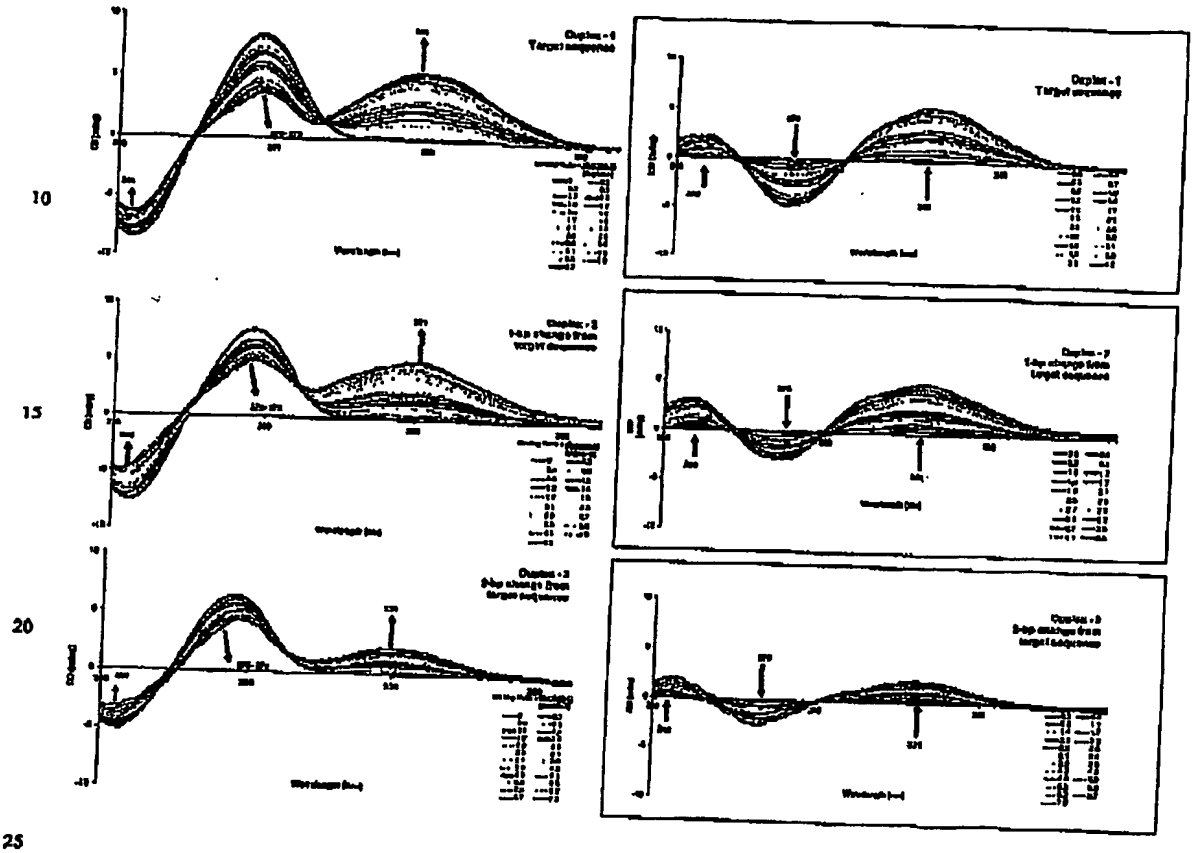


Figure 3

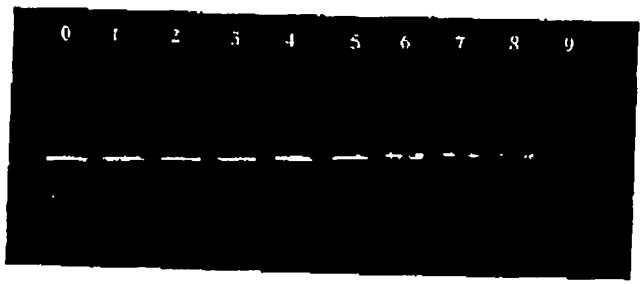


Figure 4

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/AU04/001368

International filing date: 07 October 2004 (07.10.2004)

Document type: Certified copy of priority document

Document details: Country/Office: AU
Number: 2003905512
Filing date: 07 October 2003 (07.10.2003)

Date of receipt at the International Bureau: 01 November 2004 (01.11.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse